JOURNAL OF BACTERIOLOGY, May 1974, p. 425-433
Vol. 118, No. 2

Printed in U.S.A.

Electron Microscope Analysis of Partial Denaturation of F Factor Deoxyribonucleic Acid

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Received for publication 28 January 1974

Partial denaturation pattern of sex factor deoxyribonucleic acid of *Escherichia coli* was studied by electron microscopy. Clustering of the adenine-plus-thymine-rich regions in one part of the molecule was revealed. The positions of these regions were located on the physical map of F by analyzing the partial denaturation pattern of heteroduplexes between F and F-prime factors with various parts of F sequences deleted.

In *Escherichia coli*, exchange of genetic information between chromosomes is sometimes dependent on the functions of the sex factor F in the donor cell. The F factor is a closed circular deoxyribonucleic acid (DNA) molecule with a molecular weight of 63×10^6 (6). About one-third of the sequences in F DNA are essential for fertility; about 17% of F sequences, clustered in one region of the molecule, can be deleted without affecting any known functions. The sites on F used for integration to form an Hfr, and the sites on the integrated F for excision to form an F-prime factor have all been mapped within this dispensable region for the several F-prime factors studied (5, 6; N. Davidson, personal communication).

The base composition of F is about 49% guanine plus cytosine (G + C), similar to that of the *E. coli* chromosome. The distribution of G + C content in F DNA has been shown to be asymmetrical (2). About 10% of the molecule has a G+C content of about 44%, whereas the rest has about 50% G + C. Falkow and Citarella (2) have suggested that the nonuniform base composition may have functional significance. It was proposed that the breaking point, i.e., the point of origin for conjugal transfer of the DNA, lies within the adenine-plus-thymine (A + T)-rich region. The high G + C region was shown to contain sequences homologous to *E. coli* chromosomal DNA. It was suggested that these homologous sequences are used for the integration of F into the bacterial chromosome (2).

The purpose of the present work is to map the A + T rich regions on the physical map of F (5, 6), and thereby study possible correlations between base composition and biological function.

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MATERIAL AND METHODS

Bacterial strains. The bacterial strains W1485(F), JE3513(F8-33), and ND3(FΔ(0-14.5)) and the structures of the episomes they carry have been described previously (6). KLF253(F152-1) was obtained from B. Low. The structure of F152-1 will be reported elsewhere (E. Ohtsubo and M.-T. Hsu, manuscript in preparation).

DNA isolation. The procedure of Sharp et al. (6) was used.

Partial denaturation analysis. Conditions for denaturing regions in DNA with lower G + C content (hence lower melting temperature) were achieved by raising the formamide concentration, essentially as described by Davis and Hyman (1). The DNA (0.5 μg/ml) to be studied was dialyzed against the following solution: 80% formamide, 5×10^{-2} M NaCl, 5×10^{-3} M tris(hydroxymethyl)aminomethane (Tris), 5×10^{-4} M ethylenediaminetetraacetic acid (EDTA), pH 8.5. A 50-μliter amount of the DNA solution was mixed with 1 to 2 μliters of cytochrome c solution (1 mg/ml in 0.1 M Tris, 0.01 M EDTA, pH 8.5) and then spread onto a hypophase of 50% formamide and one-tenth concentration of the electrolyte used in the spreading solution. After waiting for 2 to 3 min, the DNA was picked up with a parlodion coated copper grid stained with uranyl acetate (5×10^{-5} M in 90% ethanol) and rotary shadowed with platinum. We find that it is important to wait 2 to 3 min before picking up DNA after spreading. DNA picked up too soon shows considerable lateral aggregation. This is probably due to aggregation of cytochrome c in high formamide solvents. DNA picked up too late (after 5 min) has poorer contrast. The optimal waiting time depends somewhat on the concentration of formamide used. The higher the formamide concentration, the longer one has to wait.

Heteroduplex molecules were formed according to the procedure of Sharp et al. (6). DNA of each episome was mixed and denatured with 0.3 N NaOH, neutralized with Tris-hydrochloride, and renatured by dialyzing at room temperature for 2 h against 70%

formamide, 0.25 M NaCl, 0.1 M Tris, 0.01 M EDTA, pH 8.5.

Micrographs were enlarged and traced on a Nikon projector. The length of DNA in a denatured region was taken as the average of the lengths of the two single-stranded branches. In the case of heteroduplexes, only F sequences were measured. The sum of the lengths of denatured and native regions (and the single-stranded F DNA in the heteroduplex molecules) was normalized to unity and the length of the denatured region was expressed as a fraction of the length of F. Some of the heteroduplex molecules studied were incomplete structures, i.e., formed by renaturation between incomplete strands. The lengths of characteristic segments of the molecules are known by previous heteroduplex analysis (6). Histograms of the frequency with which a particular region became single stranded under the partial denaturation condition used were constructed by using a length interval of ($\frac{1}{250}$)F. For a heteroduplex, structural markers of the heteroduplex were used to align the structures and normalize the lengths. For the F homoduplex, the partially denatured molecules were aligned by one of the two largest and most frequently observed denaturation loops.

RESULTS

Figure 1 shows a histogram of the partially denatured regions of F DNA when spread from 80% formamide, 5×10^{-2} M NaCl, 5×10^{-3} M Tris, 5×10^{-4} M EDTA, pH 8.5, at room temperature (21 C). An electron micrograph of a partially denatured F DNA is shown in Fig. 2. Four major peaks (I, II, III, IV) and two minor peaks (V, VI) can be seen in the histogram. An interesting feature is that most of the A + T-rich sequences are clustered in one general region of F, comprising about 30% of the molecule.

To map the positions of these A + T-rich segments on the physical map of F (6), partial denaturation of heteroduplexes between F and several F-prime factors were studied. The F-prime factors used delete different portions of F sequences and therefore are useful for mapping the A + T-rich sequences. The results are described below.

F8-33/F heteroduplex. F8-33 (it was called F8(N33) in ref. 6) is a transfer-defective derivative of F8 (2, 6). F sequences between coordinates 8.5 and 16.3 are deleted in this episome as can be seen in Fig. 3a. There are also two insertion loops, *c* and $\epsilon\zeta$, mapped previously at coordinates 91.0 and 35.2 (revised coordinates, R. C. Deonier, private communication), respectively. The insertion loop *c* was chosen as a position marker to map the partially denatured regions in the F8-33/F heteroduplex. An electron micrograph of a partially denatured F8-33/F heteroduplex at the same partial denaturation condition used for F is shown in Fig. 4.

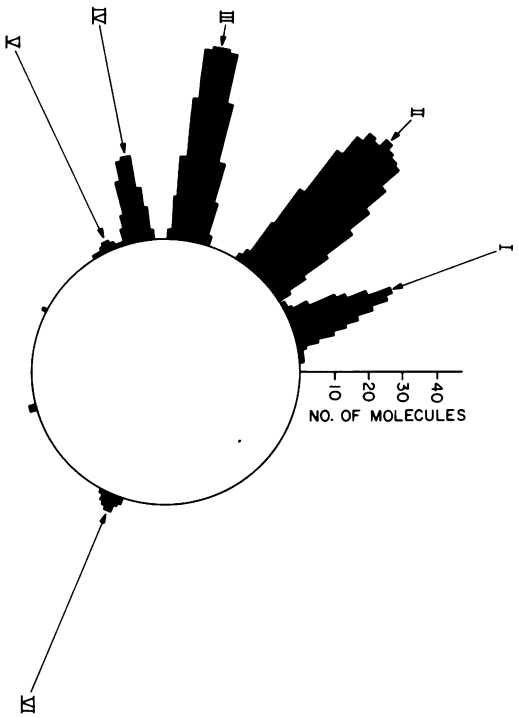

FIG. 1. *A histogram of the partially denatured regions in F factor DNA.*

The histogram of the distribution of partially denatured regions is shown in Fig. 5b. It is plotted as a linear map with coordinates 0.0 of F at the left end of the map. Five peaks at coordinates about 4, 19, 25, 30, and 63 were observed. Peak VI in Fig. 1 can be readily identified as the peak at coordinate 63 in Fig. 5b. By aligning this peak of the histogram of F and that of the F8-33/F heteroduplex, the coordinates of the other A + T-rich segments of F can be assigned as shown in Fig. 5a. This assignment is confirmed by the analysis of the following heteroduplexes.

FΔ(0–14.5)/F heteroduplex. FΔ(0–14.5) is a deletion mutant of F with the sequence between 0.0 and 14.5 deleted (see Fig. 3b). Therefore, if the previous assignment is correct, FΔ(0–14.5)/F should show only four A + T-rich regions around coordinates 19, 25, 30, and 63. This is indeed the case as shown in Fig. 5c and 6.

F152-1/F heteroduplex. The structure of the F152-1/F heteroduplex is shown in Fig. 3c. The F sequences with coordinates between 0.0 and 2.8 (revised coordinates, R. C. Deonier, private communication) are deleted in F152-1. Since the F sequence around coordinate 2.8 is probably A + T rich as shown in Fig. 5a, point *a* in

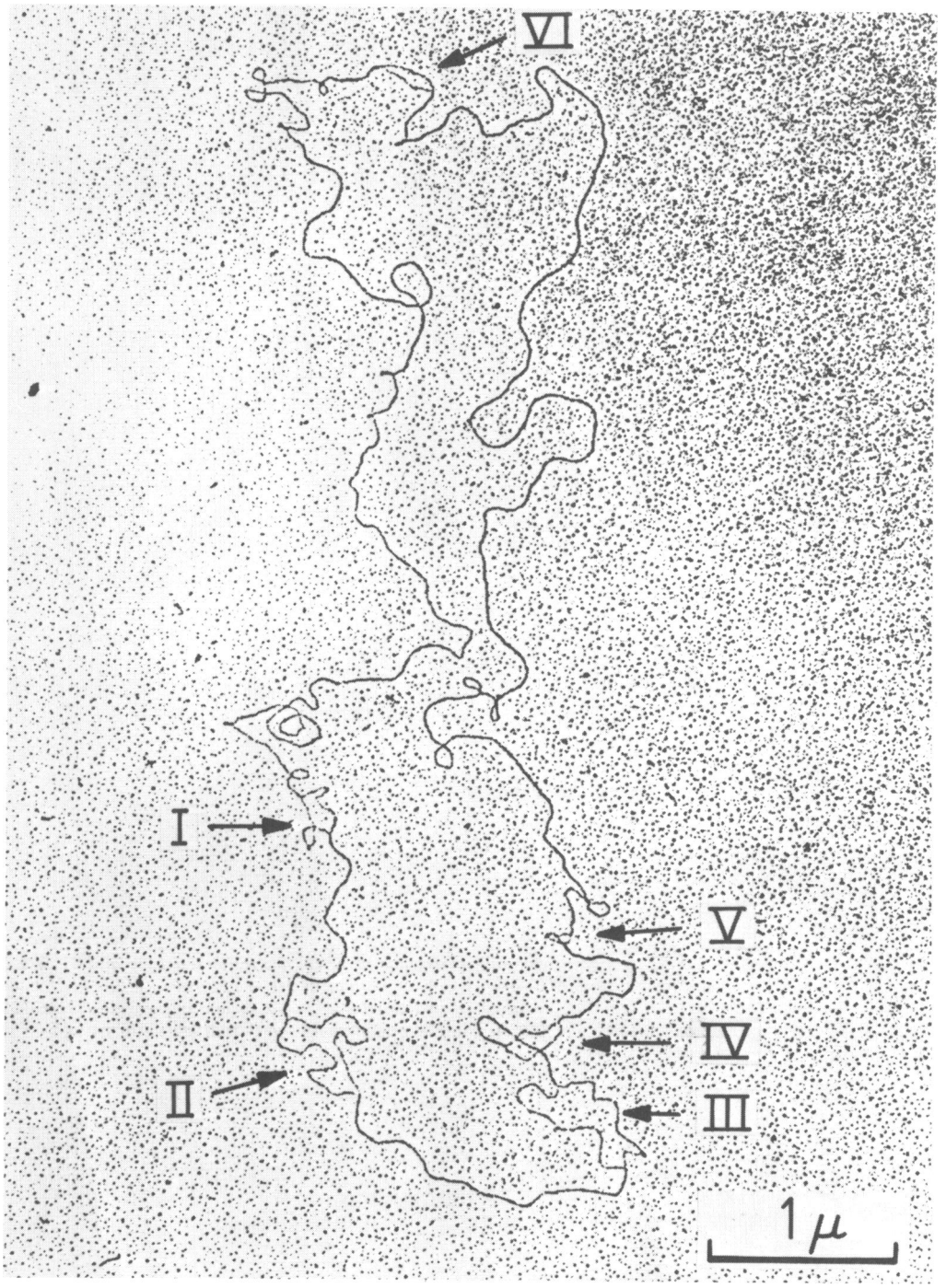

FIG. 2. *An electron micrograph of partially denatured F DNA. A histogram is shown in Fig. 1 and 3a.*

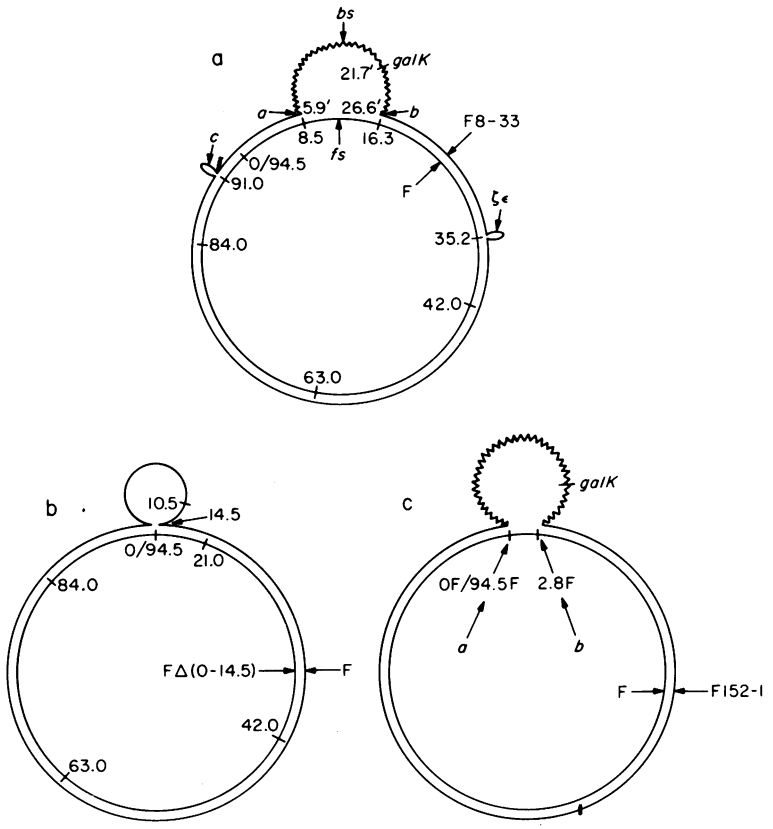

FIG. 3. *(a) Heteroduplex of F8-33/F. The two small insertions of F8-33 are indicated by c and εζ. The sequences of F between coordinates 8.5 and 16.3 are missing in F8-33. (b) Heteroduplex of FΔ(0-14.5)/F. The F sequences between coordinates 0 and 14.5 are deleted in FΔ(0-14.5) is seen as a single-strand insertion-deletion loop. (c) Heteroduplex of F152-1/F. In F152-1, the F sequence between 0 and 2.8 is substituted by bacterial DNA carrying the gal operon.*

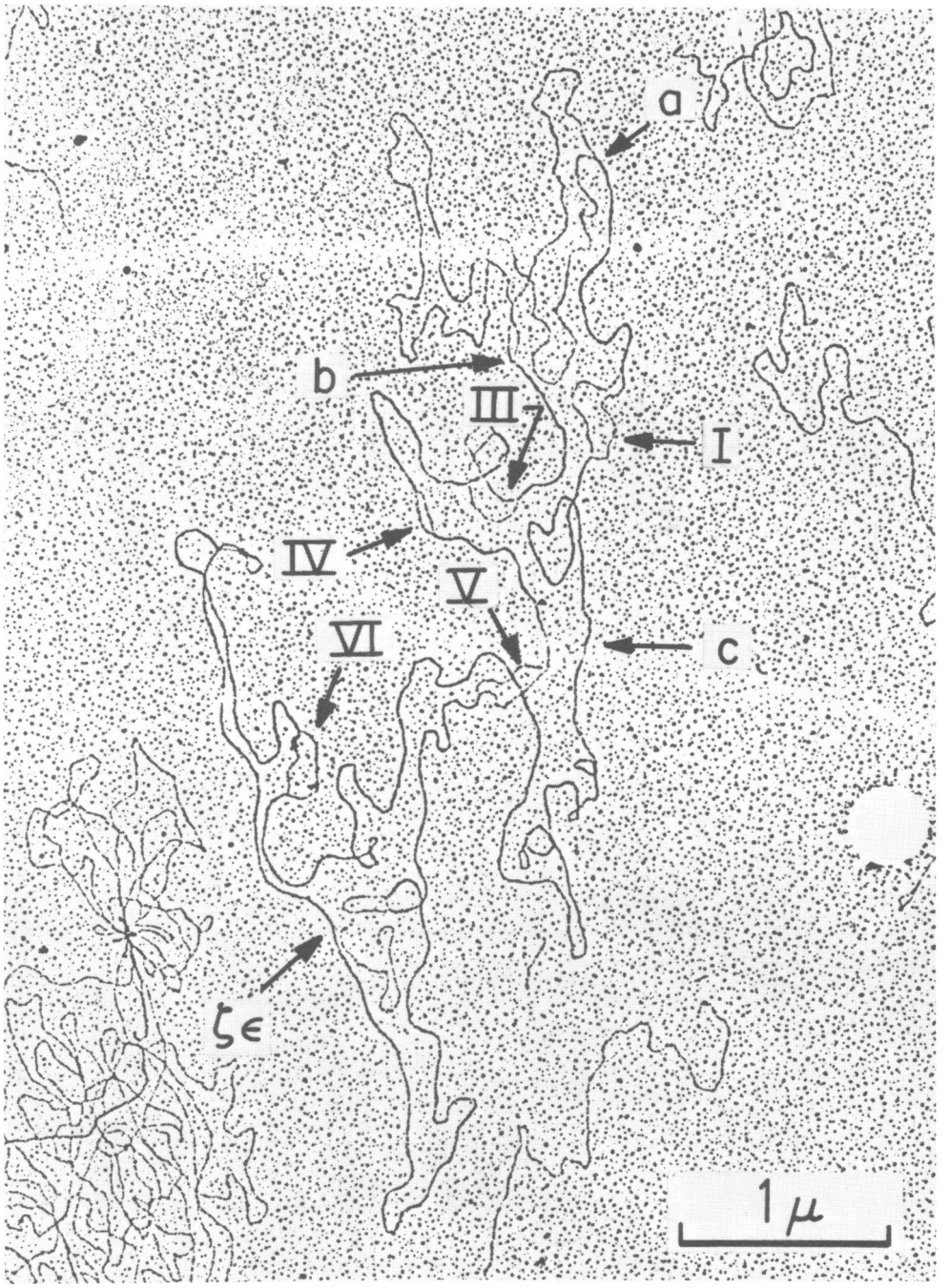

FIG. 4. *A partially denatured F8-33/F heteroduplex. The substitution loop and the two insertion loops are labeled by a and b, and c and ϵζ, respectively. Five partially denatured regions (I, III, IV, V, VI) can be seen. A histogram is shown in Fig. 3b.*

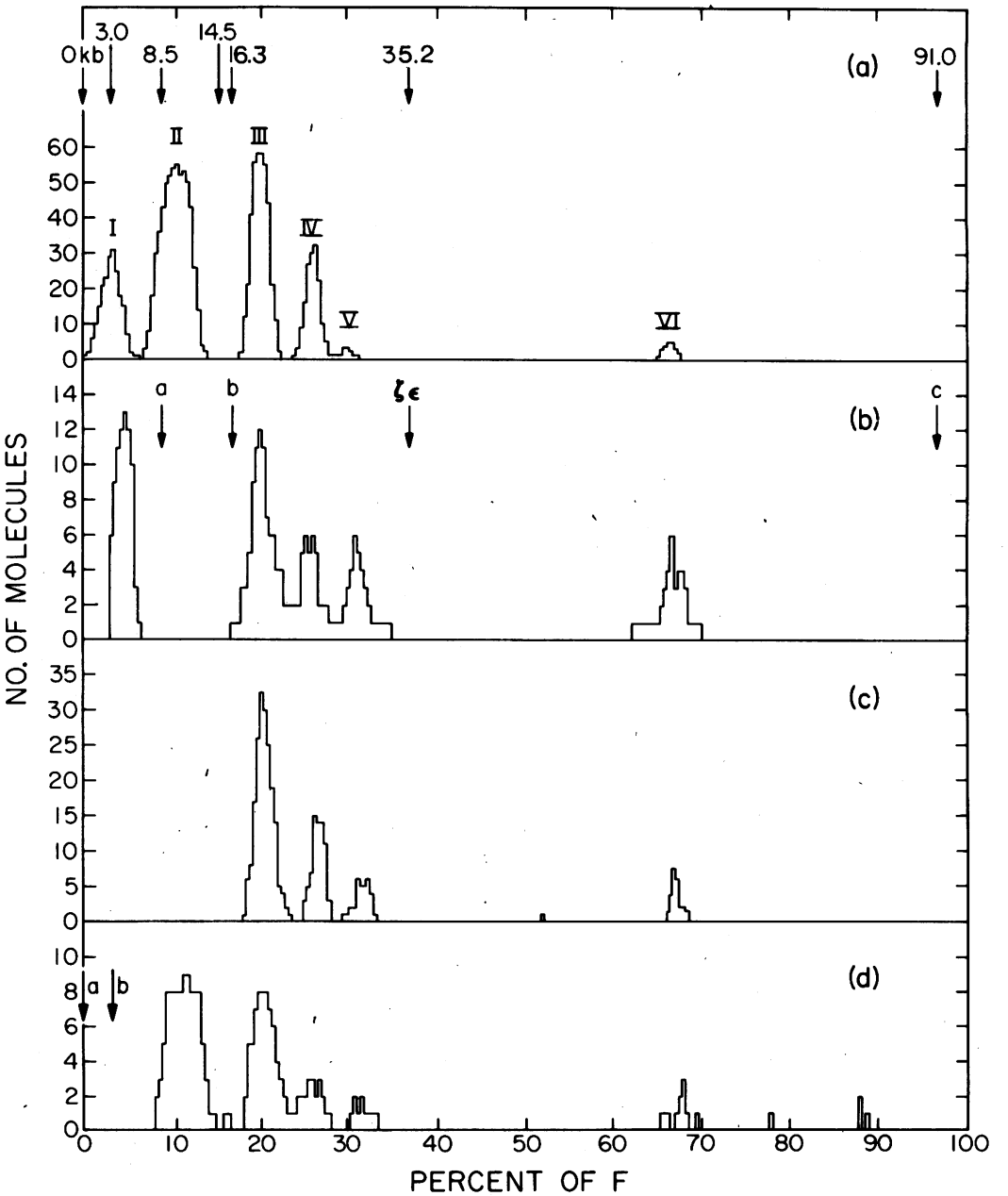

FIG. 5. *(a) Linear form of the histogram shown in Fig. 1. Several relevant coordinates of F DNA are shown in the top of the figure. (b) A histogram of the partially denatured regions in the F8-33/F heteroduplex. The labels can be used to correlate the histogram with the structure seen in Fig. 2a. (c) A histogram of the partially denatured regions in the FΔ(0-14.5)/F heteroduplex. (d) A histogram of the A + T-rich regions in F152-1/F. The substitution loop seen in Fig. 2c is labeled by a and b.*

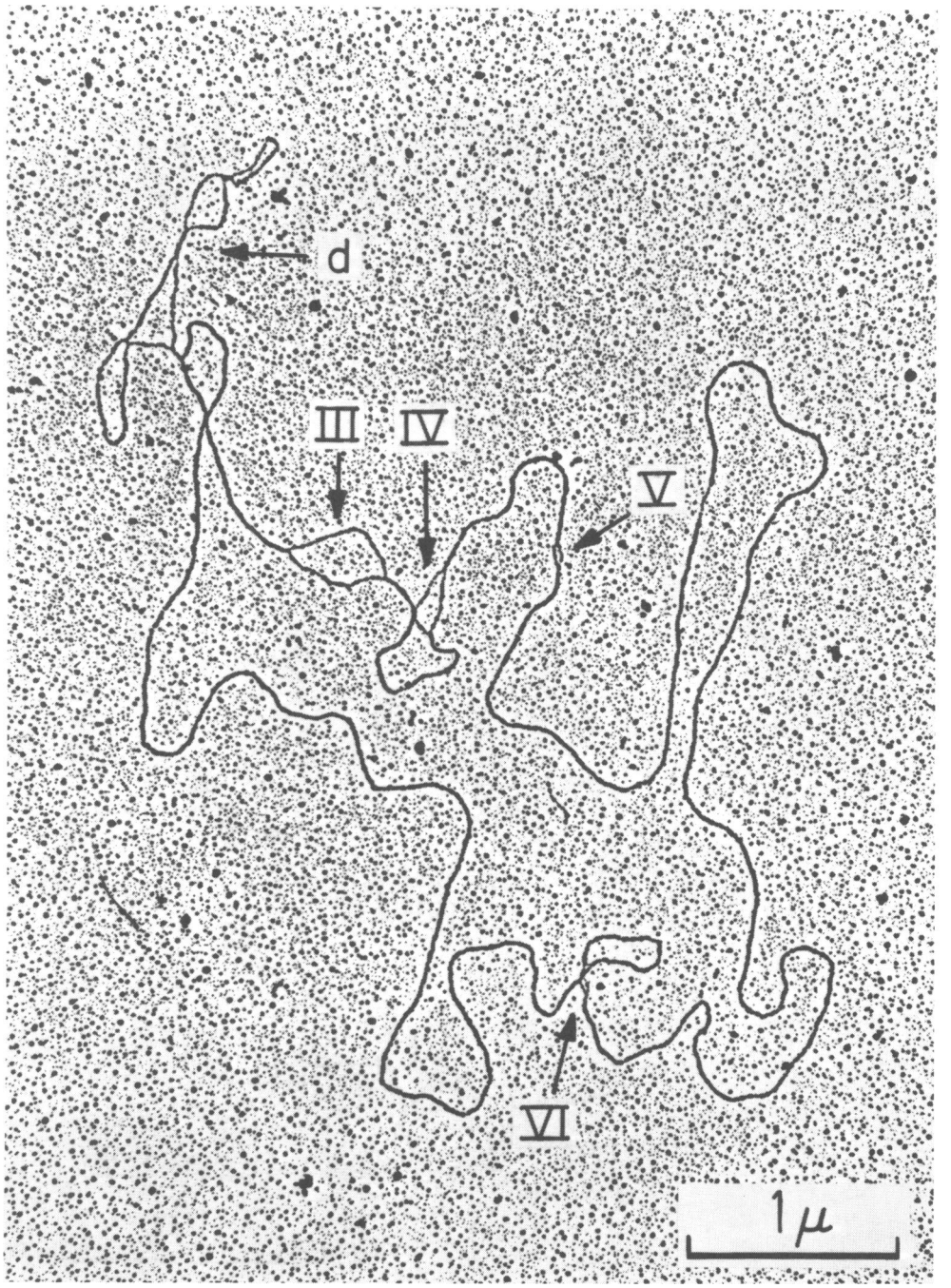

FIG. 6. *A partially denatured FΔ(0-14.5)/F heteroduplex. The single-strand deletion loop which is the F sequence deleted in FΔ(0-14.5) is indicated by the label d. Four partially denatured regions (III, IV, V, VI) are observed. A histogram is shown in Fig. 3c.*

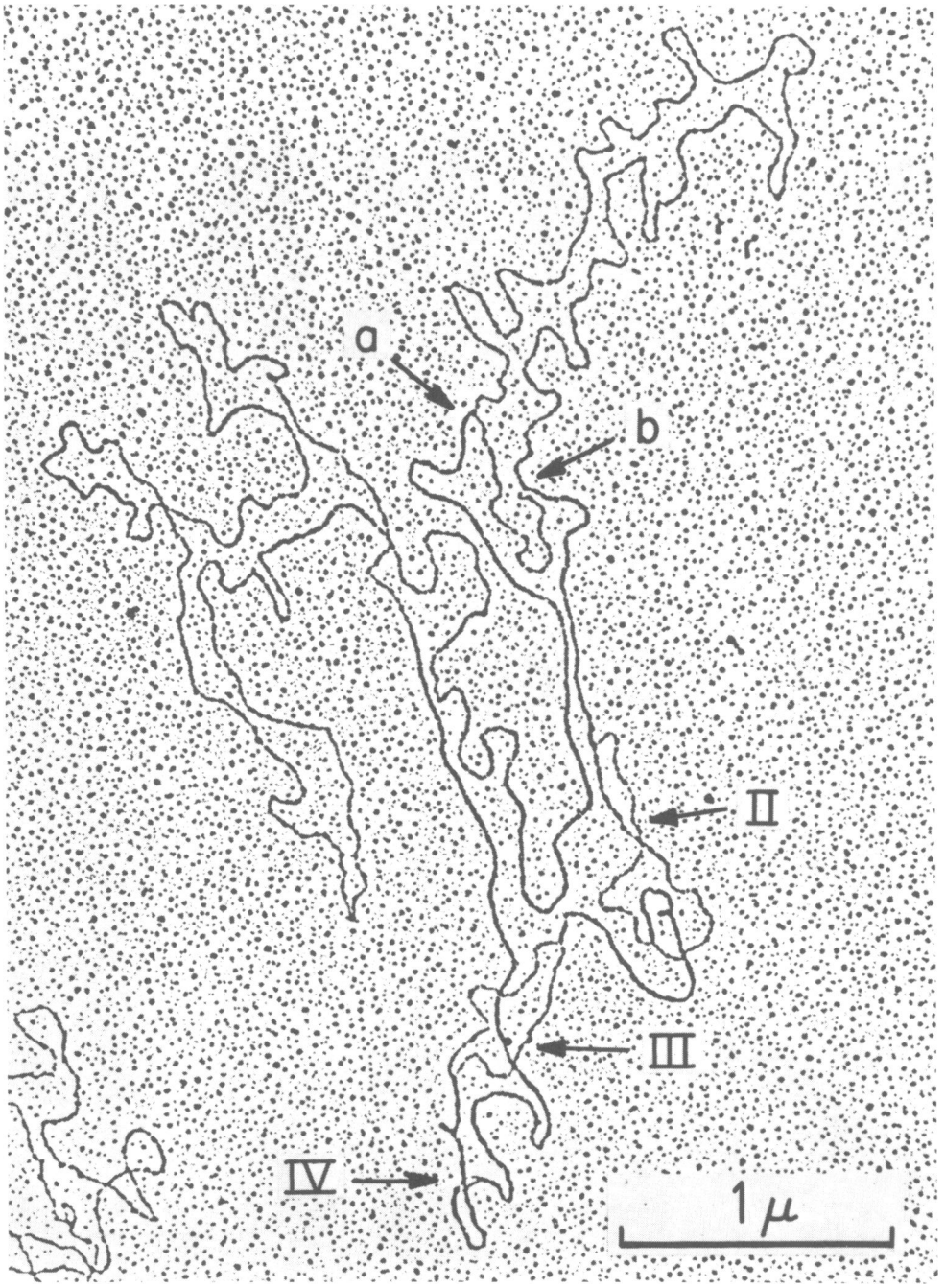

FIG. 7. *A partially denatured F152-1/F heteroduplex. The molecule shown in this micrograph is formed between incomplete strands of the episomes. Two of the five partially denatured regions, V and VI, are not shown in this micrograph.*

Fig. 3c with coordinate 0.0 is chosen as the reference point. The histogram of the partially denatured regions is shown in Fig. 5d. An electron micrograph of the partially denatured F152-1/F heteroduplex is shown in Fig. 7. Five A + T-rich regions were observed. They are mapped at coordinates around 10, 19, 25, 30, and 63, respectively. This result is consistent with the previous assignment.

DISCUSSION

The data presented above show that most of the A + T-rich sequences of F DNA are clustered in one part of the molecule, between coordinates 0 and 30. Thus the DNA sequence in this region has several interesting properties: (i) it is A + T rich as shown above; (ii) it is used for recombination with the *E. coli* chromosome for the integration or excision of F (see Table 1); and (iii) it is nonhomologous with R(fi$^+$) and colV factors. (The region of sequence homology between these plasmids and F extends clockwise from coordinate 50 to 94.5/0.0, where it ends abruptly [5].) We may speculate that the region from 0 to 30 F is bacterial DNA acquired by the "original" F factor through repeated genetic interactions.

Falkow and Citarella (2) have suggested that the A + T-rich region of F DNA may serve as the natural breaking point in bacterial conjugation. Since different portions of the F sequence between 0 and 30 can be deleted in various episomes (see Table 2) without affecting either the autonomous replication or the fertility functions, the only A + T-rich region that could possibly account for such a correlation will be the region mapped at around coordinate 63 (peak VI in Fig. 1). It is therefore interesting to note that this A + T region is located in a region between coordinates 43 and 68, which, we believe, contains the genes for autonomous replication and the structural origin for conjugal transfer. This conclusion is based on the following facts: (i) sequences clockwise from coordinate 68.0 to 94.5/0.0 can be deleted without affecting autonomous replication or transferability when complemented by R100-1 (6); and (ii) sequences between 0.0 and 43.0 can be deleted without affecting either autonomous replication or transferability (see Table 2). However, a definite correlation between the A + T-rich segment VI and the transfer origin, or the replication origin of F remains to be studied.

TABLE 1. *Integration and excision sites of various F-primes*

Episome	Integration site	Excision site	Reference
F100-1[a]	0.0	2.8	1, —[b]
F100-2	0.0	2.8	2, —[b]
F152[a]	0.0	2.8	[b]
F152-1	0.0	2.8	[b]
F42[a]	0.0	2.8	1
F8	8.5	16.3	6
F210	8.5	11.5	[c]
F14	3.0-8.5		[d]
F13	16.3-17.5		[c]

[a] F-100, F-152, and F-42 are systematic names for F$_1$*gal*, F$_2$*gal*, and F*lac* (5).

[b] E. Ohtsubo and M.-T. Hsu, manuscript in preparation.

[c] E. Ohtsubo and S. F. Hu, private communication.

[d] E. Ohtsubo, R. C. Deonier, H. J. Lee, and N. Davidson, private communication.

TABLE 2. *Sequences deleted in various F-primes*

Episome	F sequence deleted	Reference
F152	0.0-2.8	[a]
F100	0.0-2.8	[a]
F42	0.0-2.8	1
F210	8.5-11.5	[b]
F8	8.5-16.3	1
FΔ(0-14.5)	0.0-14.5	1
FΔ(33-43)	33-43	[c]
F8(P6)	0.0-8.5, 68.1-94.5	1
F13-4	16.3-37.2	[b]

[a] E. Ohtsubo and M.-T. Hsu, manuscript in preparation.

[b] E. Ohtsubo and S. F. Hu, private communication.

[c] W. M. Anthony, private communication.

ACKNOWLEDGMENTS

I am deeply grateful to Norman Davidson for advice and counsel, and for support through Public Health Service grant GM 10991 from the National Institute of General Medical Sciences.

This work was initiated during a discussion with Donald Robberson. His generosity in providing advice and unpublished data are greatly appreciated.

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